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Protein Synthesis in Abscission: The Distinctiveness of the Abscission Zone and its Response to Gibberellic Acid and Indoleacetic Acid

Lowell N. Lewis and Jagdish C. Bakhshi[1]
University of California, Riverside, California

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Abstract. **Abscission zone tissue of citrus was shown to have a higher rate of protein synthesis than tissue distal or proximal to it, based on the incorporation of leucine-1-^{14}C. The proximal tissue had the slowest rate of protein synthesis. As the tissue approached abscission, the rate of synthesis in the abscission zone decreased and the differences in rate of protein synthesis between the 3 zones almost disappeared. IAA, which delayed abscission, maintained the protein synthesis gradient between the abscission and proximal zones, but the distal zone tissue was as active in protein synthesis as the abscission zone. Differences in uptake of the leucine were also observed between different zones and treatments. Regardless of the tissue or the treatment, there was a sharp increase in uptake at the 24 hour point.**

Direct incubation of abscission zones in IAA and gibberellic acid (GA) indicated that the action of gibberellic acid on abscission is probably through a stimulation of protein synthesis, while IAA seems to act by maintaining the existing rate of protein synthesis in the cells of the abscission zone.

The potential of hormones such as auxin and gibberellin to act as effector substances (5, 16) that control repression and derepression of different parts of the genome and their involvement in abscission control (2, 12) led us to look at the effect of these hormones on the rate of protein synthesis in the tissue associated with abscission. This paper demonstrates the biochemical distinctiveness with respect to protein synthesis of the abscission zone from the adjacent tissue.

Materials and Methods

Seedlings of sweet orange (*Citrus sinensis*, Osbeck) were the source of the plant material used in these experiments. Plants about 9 months old were selected for uniformity of height, leaf color, and leaf quality. The growing terminal was removed so that only mature leaves were used. The leaf blade and petiolar wings were removed, leaving a 4 mm portion of the leaf midrib (the debladed midrib) attached to the petiole (12). Preliminary experiments showed no correlation between the position of the debladed midrib on the seedling and the order in which they abscised. Hormones to be tested were dissolved in 0.1 M citrate buffer, pH 6.0, and incorporated into an equal volume of 2% (w/v) agar. A 5 μliter droplet of the agar gel, containing the test compound was applied to the distal end of each explant with a tuberculin syringe. Control plants were treated with a similar drop of buffered 1% (w/v) agar. Treated seedlings were retained in the growth chamber in the dark at 25° and a relative humidity of 60% or higher.

Incorporation of L-leucine-1-^{14}C (specific activity 180 μc/mg) into the alcohol and oxalate insoluble fraction was used as a measure of the rate of protein synthesis associated with various stages of abscission. In 1 series of experiments, abscission zone sections about 1 mm thick were incubated in 2 ml of 0.1 M phosphate buffer, pH 6.0, containing the leucine-1-^{14}C and the hormone. In the second series, the hormones were applied directly to the debladed midribs on the sweet orange seedlings. At subsequent time intervals, the rates of protein synthesis of 1 mm tissue sections from the abscission zone, the proximal zone or the distal zone were determined by incubating sections in the buffer, containing leucine-1-^{14}C.

Incubation, extraction, and isotope counting procedures were the same for all samples. The labeled leucine incubation was done in the dark at 30° for 3 hours in a metabolic shaker. Twenty-five tissue sections were used per vial and there were 4 replications of each treatment. The conditions of incubation were based on preliminary experiments which showed good linearity of leucine uptake and incorporation for about 4 hours.

At the end of the incubation period, the incubation mixture was syphoned off, and the tissue sections were washed twice with 10 ml of ice-cold distilled water to remove the exterior leucine. The sections were then placed in boiling 80% (v/v)

[1] Present address: Punjab Agricultural University, Hissar, India.

methanol for 10 minutes to stop enzymatic activity and to remove absorbed but unincorporated leucine-1-^{14}C. The alcoholic extraction was repeated 3 times and the extracts pooled. Radioactivity in the alcohol soluble fraction is referred to as "uptake" in the results. The tissue was further extracted in 0.5 % (w/v) ammonium oxalate for 30 minutes at 100° and washed twice with distilled water to remove pectins and to insure that the amino acid incorporation was not just amino acids bound to pectin. No appreciable counts were ever detected in the oxalate soluble fraction.

The oxalate insoluble material was transferred to a counting vial containing one-half ml of distilled water and 5 ml of a solution containing 100 g of naphthalene, 10 g of 2,5-diphenyl oxazole (PPO), and 0.25 g of 1,4-*bis* [2-(methyl-5-phenyloxazolyl)-benzene] (dimethyl POPOP) per liter of dioxane, and the radioactivity was measured in an Ansitron liquid scintillation counter. This radioactivity was taken as a measure of the incorporation of leucine into protein. These data are presented in the text as the percent of leucine incorporated into protein and were calculated by dividing the counts in the protein fraction by the sum of the counts in free amino acids plus protein and multiplying by 100.

To insure that the counts measured in the alcohol and oxalate insoluble fraction represented the incorporation of leucine into protein, 3 experiments were done: A) Increasing quantities of cold leucine in the incubation mixture caused a proportional decrease in the incorporation of labeled leucine. B) A solution of 1 % (w/v) fungal protease in 0.1 M citrate buffer, pH 6.0, solubilized 60 and 99 % of the radioactivity in 24 and 40 hours, respectively. The incubation mixture was covered with a layer of toluene to minimize bacterial infection. C) Following acid hydrolysis in 6 N HCl and freeze drying of the hydrolysate, 90 % of the radioactivity co-chromatogrammed with leucine in butanol, acetic acid and water (9:1:2.5, v/v) on Whatman No. 3 paper.

After counting, the tissue was recovered from the scintillation mixture, dried, and weighed. All counts were based on the dry weight of alcohol and oxalate insoluble tissue. This was done for ease of operation, as excessive handling at the time of preparation increased variability.

Results

Leucine-1-^{14}C Uptake and Incorporation into Protein. The most striking change in uptake was observed during the first 24 hours after deblading (fig 1). Abscission zone tissue placed in leucine-^{14}C at the time of deblading (zero time) took up 611 cpm per mg of tissue in the free amino acid fraction, but the abscission zone from petioles which had been debladed for 24 hours took up 1765 cpm. Seventy-two hours after deblading, the rate of uptake had returned to values approximating those of zero time. The uptake pattern with time was the same for tissue from the distal and proximal zones as observed for the abscission zone; however, the actual values were about 40 % less in the proximal zone than in the other two.

The change in rate of uptake (fig 1-3) due to hormone treatment was not evident at 24 hours, but by 72 hours the uptake pattern was different for control, GA, and IAA treated tissue. GA treated tissue, which was the closest to time of abscission (11), showed the least difference in uptake between proximal, abscission and distal zones (762, 867, and 686 cpm, respectively) while IAA treated tissue, which would not abscise for several weeks (12), showed low uptake values for proximal and distal zones, 562 and 506 cpm, respectively, but the abscission zone had the highest value of any tissue at this time—1232 cpm. The GA treatment consistently accelerated abscission by about 1 day compared with debladed controls, but IAA delayed abscission by more than 26 days (12).

Rates of leucine incorporation into protein showed several important general differences. At zero time the greatest rate of protein synthesis was in the abscission zone; 56.3 % of the leucine taken up was incorporated into protein. Distal tissue incorporated only 14.6 % and proximal tissue 2.2 % (fig 1). At the 24-hour point, regardless of treatment, there was a small drop in rate of protein synthesis in the abscission zone and an increase in both proximal and distal zones. In spite of these changes, rates of protein synthesis remained highest in the abscission zone and lowest in the proximal zone.

Hormonal effects on leucine incorporation were not evident until the 72-hour point. In the GA tissue (fig 2), the rate of protein synthesis in the abscission zone dropped sharply; in the distal zone it dropped slightly, and in the proximal zone, it increased. As a result, the rate of protein synthesis was almost the same in all 3 zones from GA treated tissue after 72 hours.

Control midribs (fig 1), which were at least a day farther from abscission than those treated with GA, had rates of protein synthesis similar to the GA in abscission and distal zones, but lower than GA in the proximal zone.

IAA (fig 3) had the most striking effect. These petioles did not abscise for several weeks and their pattern of protein synthesis was quite different than that found in petioles approaching abscission. The rate of protein synthesis in the abscission zone remained high—49.5 % of the leucine being incorporated into protein. The rate in the proximal tissue remained low (11.4 %). The rate of protein synthesis in the distal tissue rose to a value about the same as the abscission zone.

It would appear that a relatively high rate of protein synthesis in the abscission zone and low rate in the proximal zone is typical of a non-abscising system.

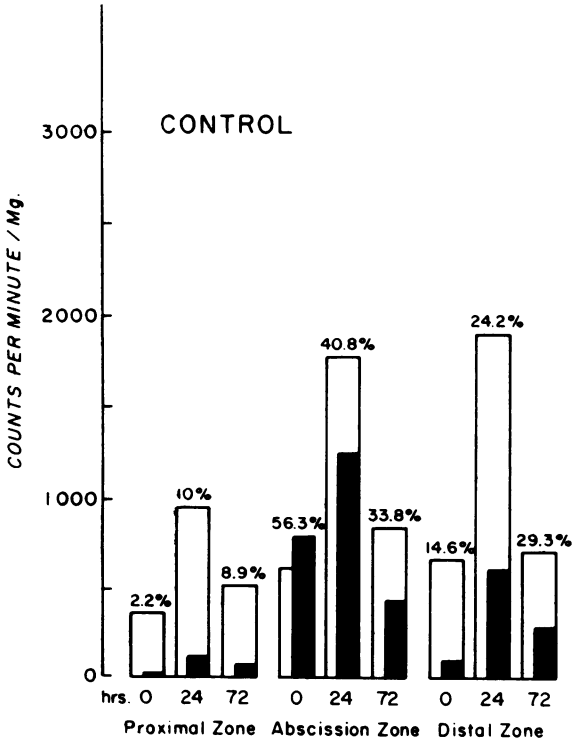

FIG. 1.

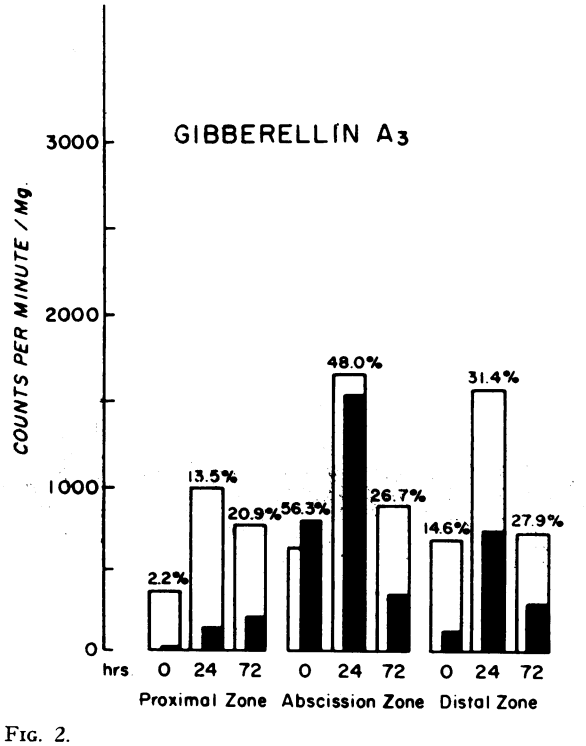

FIG. 2.

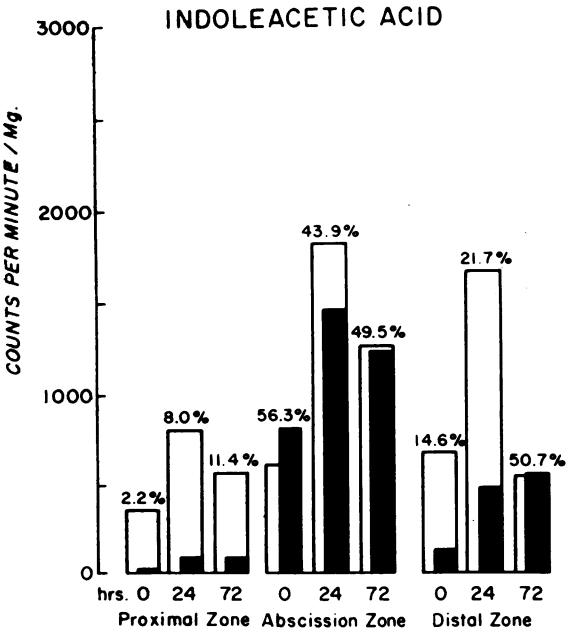

FIG. 3.

FIG. 1. (Upper left), 2. (Lower left), 3. (Above). Protein synthesis by the proximal zone, abscission zone, and distal zone of petioles of citrus leaves debladed for the indicated periods of time. The open bar represents the ^{14}C-leucine uptake. The closed bar is the ^{14}C counts incorporated into protein. Percentage values represent the percent of all ^{14}C counts present in protein and were calculated by dividing ^{14}C counts in protein by the ^{14}C counts in protein plus those in the alcohol soluble fraction $\times$ 100.

An experiment on rates of protein synthesis associated with delayed applications of IAA emphasizes the relationship between rates of protein synthesis and abscission. Petioles were treated with IAA 56 hours after deblading. Sixteen hours later (72 hrs after deblading), the rate of protein synthesis was typical of that shown for controls at 72 hours (fig 1). By the fifth day from deblading, 63 % of the IAA treated petioles had abscised. Those that abscised were the ones irreversibly committed to abscission by the time the IAA reached the abscission zone. The other 37 % were not irreversibly committed, so IAA was able to arrest the abscission process in these (12). On the ninth day after deblading, the intact petioles were sectioned and incubated in leucine-^{14}C. Their rate of protein synthesis was typical of that seen for IAA at the 72 hour point (fig 3). Therefore, even though it seemed that protein synthesis had dropped as if these petioles were approaching abscission, IAA could restore those petioles which had not passed some unknown point, to a non-abscising state typified by a high rate of protein synthesis.

There is a possibility that changes in the natural pool sizes of leucine account for most of the effects

seen in this work. We have not studied this possibility in sufficient detail to say it is of no importance, but preliminary data indicate no effect on conclusions we have drawn. There was quite an increase in the level of free leucine at the 48-hour point and corresponding drop in the percent of leucine-^{14}C incorporated. This increase in free leucine at 48 hours did not seem to have any dependence on the type of tissue or treatment. Future work will be done on leucine and other amino acids as they change with abscission.

Direct Incubation in Hormones. Direct incubation of the abscission zone in gibberellin (GA) increased the rate of protein synthesis by almost 40 % at the lowest concentrations tested (table I). As the GA concentrations increased, the rate of protein synthesis dropped until, at the highest concentration, it seemed to be lower than observed in control abscission zones. GA did not affect the rate of leucine uptake as measured by ^{14}C counts in the 80 % MeOH.

Table I. *Effect of Gibberellic Acid (GA) on the Incorporation of ^{14}C Leucine into the Abscission Zone During a 3 Hour Incubation*

Conc of GA	Oxalate insoluble material[1] Incorporated into protein	Extracted in MeOH
mg/l	*cpm/mg*	*cpm/mg*
400	508^{x}	537^{z}
40	685^{y}	534^{z}
4	711^{y}	454^{z}
0.4	900^{z}	462^{z}
0.04	878^{z}	474^{z}
Control	645^{xy}	455^{z}

[1] Values within a column which do not have a common letter are significantly different at the 5 % level. Each value is a mean of 4 individual determinations.

The effect of IAA on protein synthesis (table II) in the abscission zone seemed to follow the classical bell-shaped curve for auxin action on root, stem and bud development (18). There was an increased rate of synthesis up to about 40 ppm of IAA, but the highest concentration (400 ppm) resulted in the lowest rate of protein synthesis for any IAA concentration. The 400 ppm (2.3×10^{-3} M) concentration significantly increased the rate of leucine uptake over the control and all other IAA treatments. This level of IAA seemed to cause effects biochemically distinct from the effects seen for the other more physiological IAA concentrations.

Quantitation of Uptake and Transport of IAA and GA. To estimate the amount of hormone entering the abscission zone in direct incubation compared with the amount entering when debladed midribs were treated on the seedling, a study was done with IAA-1-^{14}C (183 μc/mg) and GA-^{3}H (60 μc/mg). Based on radioactive counts, the amount of IAA and the amount of GA found in the abscission zone of debladed midribs treated with 1 μg of hormone was about the same as the amount found there following direct incubation in 0.04 to 0.4 ppm of the corresponding hormone.

Table II. *Effect of Indole Acetic Acid (IAA) on the Incorporation of ^{14}C Leucine into the Abscission Zone During a 3 Hour Incubation*

Conc of IAA	Oxalate insoluble material[1] Incorporated into protein	Extracted in MeOH
mg/l	*cpm/mg*	*cpm/mg*
400	383^{y}	715^{z}
40	661^{z}	371^{x}
4	639^{z}	331^{x}
0.4	588^{xz}	406^{x}
0.04	580^{xz}	344^{x}
Control	441^{xy}	416^{x}

[1] Values within a column which do not have a common letter are significantly different at the 5 % level. Each value is a mean of 4 individual determinations.

The counts present in the tissue were not verified as still being associated with the corresponding hormone, so the actual amount of hormone transported to the abscission zone may be lower than the counts indicated but it would not be any higher.

Discussion

A factor in this data which complicated understanding was the direct effect of leaf removal on the biochemistry of the entire petiole. Regardless of the treatment, within 24 hours, there were large changes in the uptake capacity and rates of protein synthesis in the distal, abscission, and proximal zones. These changes may be a wounding response, they may result from the decrease in photosynthate supply due to leaf removal, or they may be due to an increase in the petiole or root supplied materials which the leaf would normally receive. (These petioles were still attached to the plant.) Because of the differences in uptake, incorporation of ^{14}C-leucine was expressed as a percent of total uptake. If ^{14}C-leucine is considered strictly as total counts per minute incorporated regardless of uptake, most of the conclusions would be the same, but there are 2 notable exceptions. In the abscission zone at 24 hours for all treatments, total counts per minute incorporated doubles at 24 hours compared to zero time but the percent incorporation declines slightly. The IAA treated distal tissue at 72 hours shows a doubling over the analagous treatment at 24 hours by percent uptake but little change as total counts per minute incorporated. It will not be known which of these conclusions is physiologically correct until more is known about the systems controlling uptake and incorporation.

One of the striking features of these data, with or without the leaf removal effect, was the distinct difference in rates of leucine uptake and protein synthesis among sections of tissue from distal, abscission, and proximal zones. The abscission zone was clearly distinct from the other tissues in its initial rate of protein synthesis. Maintenance of a sharp difference in uptake and protein synthesis between the abscission and proximal zones seemed to be typical of non-abscising tissue. Tissue close in time to abscission seemed to be typified by little, if any, difference between uptake and protein synthesis in the 3 zones. Similar differences have been suggested by others. Biggs and Leopold (3, 4) suggested that the primary action of auxin was on the abscission zone itself, and that proximal and distal tissue only affect the intensity of action. Osborne (15) and Yager (19) reported gradients of pectin methyl esterase (PME) activity across the abscission zone in intact tissue and noted that PME activity in the abscission zone decreased as abscission approached. In Osborne's system, when PME activity was higher in the petiole than the abscission zone, abscission followed. LaMotte *et al.* (11) observed similar changes in PME during abscission. Addicott (2) concluded that GA acted directly on the abscission zone. Although the present status of understanding does not allow us to assign specific functions in abscission control to different tissue zones, it does seem clear that there are sharp differences in the biochemistry of tissue from proximal through abscission to distal zones. Perhaps gradient (1, 2) and concentration (4, 10) theories should be reexamined in the light of this naturally existing biochemical gradient.

How gibberellin accelerates and auxin retards the abscission process relative to the biochemical gradient is the fundamental question. From the experiments where the abscission zones were incubated with the hormone plus labeled leucine, it was clear that low levels of GA (0.04–0.4 ppm) stimulate protein synthesis. The same low concentrations of IAA did not significantly stimulate protein synthesis. According to the experiments with labeled IAA and GA, the application of 1 μg of either hormone to the cut end of the debladed midrib supplied about the same amount of hormone to the abscission zone as direct incubation in 0.04 to 0.4 ppm of the corresponding hormone. Since 1 μg of GA accelerates abscission and 1 μg of IAA retards abscission, it was concluded that the GA acceleration depends on an increase in the rate of protein synthesis while the IAA delay depends primarily on a maintenance of the existing enzyme systems with little or no increase in the rate of protein synthesis. In this tissue the action of GA cannot be ascribed to an increase in ethylene production since 1 μg of GA does not cause any increase in ethylene production over controls of citrus leaf explants. However, 1 μg of IAA does cause an increase in ethylene production in citrus explants (13). In the direct incubation studies, the levels of IAA at 4 ppm and above were sufficiently high to act via ethylene production, but these were incubated for only 3 hours which seems too short for an ethylene effect (6).

Edelman and Hall (9) demonstrated a system with invertase in artichoke tuber disks which may be analagous here. They showed that GA enhanced DNA dependent invertase activity and that IAA reduced the amount of invertase activity. They reasoned that auxin repressed the genes for invertase formation, while GA derepressed them. It is well established that GA induces *de novo* synthesis of α-amylase and several other hydrolytic enzymes (7, 8, 19, 20), all of which could be important in abscission. Therefore, it seems logical that GA could accelerate abscission by inducing or increasing enzyme synthesis.

It may be more difficult to accept that IAA prevents abscission by maintaining the cell as it was, *i.e.*, not allowing the abscission and/or senescence enzymes to be activated. Such a role for auxin has been indicated by other workers. Sacher (17) showed that in the absence of exogenous auxin, 20 % of the RNA in excised bean pod tissue was degraded in 15 hours and then DNA and ribosomal protein degradation began. He concluded that the primary effect of auxin in preventing senescence was to maintain the level of RNA. Osborne (16) also presented evidence that 2,4-D could retard senescence in *Prunus* leaves by maintaining levels of proteins in aging leaves. Ockerse, Siegel, and Galston (14) reported the appearance of an eighth peroxidase isozyme as dwarf pea tissue aged. The appearance of the isozyme in excised sections was repressed by IAA. Yung and Mann (22) demonstrated that Barban could inhibit GA action in inducing α-amylase synthesis and they concluded that Barban inhibits the onset of changes, but it does not inhibit on-going processes. It has been shown (12) that Barban inhibits the abscission accelerating effect of GA but it does not alter the IAA retarding effect. Therefore it seems that IAA delays abscission of debladed midribs by maintaining the cell in its pre-debladed state.

Literature Cited

1. Addicot, F. T. 1961. Auxin in the regulation of abscission. In: Handbuch der Pflanzenphysiologie. Springer-Verlag, Berlin. XIV: 828–38.
2. Addicot, F. T. 1965. Physiology of Abscission. In: Handbuch der Pflanzenphysiologie. Springer-Verlag, Berlin. XV/II: 1094–1126.
3. Biggs, R. H. and A. C. Leopold. 1957. Factors influencing abscission. Plant Physiol. 32: 626–32.
4. Biggs, R. H. and A. C. Leopold. 1958. The two phase action of auxin on abscission. Am. J. Botany 45: 547–51.
5. Bonner, J. 1965. The Molecular Biology of Development. Oxford University Press, Oxford. 155 p.

6. Burg, S. P. and E. P. Burg. 1966. The interaction between auxin and ethylene and its role in plant growth. Proc. Natl. Acad. Sci. US 55: 262–69.
7. Chandra, G. R. and J. E. Varner. 1965. Gibberellic acid controlled metabolism of RNA in aleurone cells of barley. Biochem. Biophys. Acta 108: 583–92.
8. Chrispeels, M. J. and J. E. Varner. 1967. Gibberellic acid-enhanced synthesis and release of α-amylase and ribonuclease by isolated barley aleurone layers. Plant Physiol. 42: 398–406.
9. Edelman, J. and M. A. Hall. 1964. Effect of growth hormones on the development of invertase associated with cell walls. Nature 201: 296.
10. Gaur, B. K. and A. C. Leopold. 1955. The promotion of abscission by auxin. Plant Physiol. 30: 487–90.
11. LaMotte, C. E., J. R. Mathur, and B. Peter. 1965. Changes in pectin methyl esterase activity associated with leaf abscission in coleus. Plant Physiol. 40: xxvii.
12. Lewis, L. N. and J. C. Bakhshi. 1968. Interactions of indoleacetic acid and gibberellic acid in leaf abscission control. Plant Physiol. 43: 351–58.
13. Lewis, L. N., R. L. Palmer, and H. Z. Hield. 1968. Interactions of auxins, abscission accelerators, and ethylene in the abscission of Citrus fruit. Proc. Plant Growth Substances Intern. Conf. 6th: In press.
14. Ockerse, R., B. Z. Siegel, and A. W. Galston. 1966. Hormone-induced repression of peroxidase isozyme in plant tissue. Science 151: 452–54.
15. Osborne, D. J. 1958. Changes in distribution of pectin methyl esterase across leaf abscission zones of *Phaseolus vulgaris*. J. Exptl. Botany 9: 446–57.
16. Osborne, D. J. 1965. Interactions of hormonal substances in the growth and development of plants. J. Sci. Food Agr. 16:1–13.
17. Sacher, J. A. 1965. Senescence: Hormonal control of RNA and protein synthesis in excised bean pod tissue. Am. J. Botany 52: 841–48.
18. Thimann, K. V. 1937. On the nature of inhibitions caused by auxins. Am. J. Botany 24: 407–12.
19. Varner, J. E. 1964. Gibberellic acid controlled synthesis of α-amylase in barley endosperm. New Phytologist 39: 362–69.
20. Varner, J. E. and G. R. Chandra. 1964. Hormonal control of enzyme synthesis in barley endosperm. Proc. Natl. Acad. Sci. US 52: 100–06.
21. Yager, R. E. 1960. Possible role of pectic enzymes in abscission. Plant Physiol. 35: 157–62.
22. Yung, K. and J. D. Mann. 1967. Inhibition of early steps in the gibberellin-actuated synthesis of α-amylase. Plant Physiol. 42: 195–200.